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[COMMUNICATION]

A Morphometric Study on Pituitary Pars Intermedia Cells of Male Mice Fed a Liquid Diet Supplemented with Urea

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ABSTRACT—An electron microscopic and morphometric study on pars intermedia (PI) cells of the pituitary was undertaken in male mice given either a liquid diet consisting of 3% powdered milk in a 5% glucose solution or the identical liquid diet supplemented with 1% urea for 10 days. Three parameters representing the secretory activity of PI cells were examined. The percent area of the cytoplasm occupied by the rough endoplasmic reticulum (an index of protein synthesis) increased to 124% and 172% of the control value in mice maintained on the liquid diet alone and on the liquid diet containing 1% urea, respectively; the numerical density of immature Golgi granules (an index of granule formation) also increased to 143% and 185% of the control value in those given the liquid giet alone and the liquid diet supplemented with 1% urea, respectively. In contrast, the numerical density of secretory granules (an index of granule storage) decreased to 74% and 62% of the control value in animals fed the liquid diet alone and the liquid diet supplemented with 1% urea, respectively. These results indicate that the balance between synthesis and release of PI hormones is in equilibrium at a higher level in mice fed the liquid diet with urea than in animals given the liquid diet without urea.

INTRODUCTION

Our previous morphometric studies on the pars intermedia (PI) of the mouse pituitary have demonstrated that dietary sodium restriction induced hypersecretion in pituitary PI cells [1], and that pharmacological impairment of the renin-angiotensin-aldosterone system enhanced the secretory activity of PI cells in sodium depleted mice [2]. These results have led to the assumption that the PI hormone(s) is a pituitary factor that stimulates aldosterone secretion from adrenals in sodium depleted mice [3]. Further, a new method for inducing excess drinking was developed in our laboratory. Stimulation of the secretory activity of PI cells accompanied the copious drinking [4]. A recent study [5] showed that oral urea administration augented the secretory activity of PI cells in lactating mice under conditions whereby copious drinking was induced [4]. The hypersecretory activity of PI cells induced by oral urea intake in suckling dams might result from either induced drinking or excess drinking characteristic of lactating mice [5]. Thus, male mice were used in the present study and the secretory activity of PI cells was evaluated morphometrically in animals given a liquid diet supplemented with or without urea.

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MATERIALS AND METHODS

Male mice of the Jcl:ICR strain at 5 weeks of age were caged individually at 22 ± 1°C under 12 hr of light daily (07:00–19:00) with free access to food and tap water. They were divided into three groups, each consisting of 9 animals. The first group served as a control, receiving pelleted food and distilled water. The second group was sub-

jected to copious drinking. Animals were fed a liquid diet consisting of 3% powdered milk in a 5% glucose solution, and the pelleted food and distilled water were withheld. To maintain mice in an excess drinking state and under minimum nutritional conditions, 3% powdered milk was dissolved in a 5% glucose solution. The third group received the same liquid diet supplemented with 1% urea. The body weight was recorded every day and the daily consumption of the liquid diet with or without urea was expressed as % volume/body weight. After treatment for 10 days, the animals were killed by decapitation, and blood was collected from the trunk vessel. The levels of hematocrit (%) were determined, and plasma osmolality (mOsm/kg) was measured with a Shimazu OSM-1 Osmometer. The pituitaries were removed immediately after sacrifice and parasagittal slices of the specimens were fixed in a mixture of 1% glutaraldehyde and 1% paraformaldehyde (pH 7.4) for 1 hr followed by postfixation with 1% OsO_4 (pH 7.4) for another 1 hr. After dehydration the specimens were embedded in Quetol 812.

For morphometric analysis 6 of 9 animals were used in each group. Approximately 60 cells on the electron micrographs at a magnification of 10,000× were chosen at random from each group. The area of the cytoplasm and the Golgi region were measured with a planimeter (X-PLAN 360, Ushikata, Tokyo). The number of secretory granules and immature Golgi granules per unit area (μm^2) was expressed as the numerical density of the granules. The area of the rough endoplasmic reticulum (r-ER) was determined with PLANIMAX 25 (Nihon Regulator, Japan) and was expressed as the percent area of the cytoplasm oocupied by the r-ER. Statistical significance of the mean was assessed by Student's *t*-test.

RESULTS

1. *Daily liquid consumption and body weight*

In the control male mice, the daily consumption of distilled water was 27±8% v/w. In sharp cpntrast, the liquid intake increased from 121±7% v/w on day 1 to 200±12% v/w on day 10 in animals given a liquid diet (3% powdered milk in a 5% glucose solution) without urea. In the animals fed the liquid diet supplemented with 1% urea, the oral intake on day 1 was 117±8% v/w, increasing to 241±24% v/w on day 10 (Fig. 1). The body weight of the control mice was 33.2±0.7 g at 5 weeks of age and 39.3±1.2 g at 10 days thereafter. The body weight declined gradually to 76.4% of the control value on day 10 in mice maintained on the liquid diet alone, and to 76.9% in those fed the liquid diet containing 1% urea (Fig. 2).

2. *Hematocrit and plasma osmolality*

In the treated groups, hematocrit values were significantly higher than those in the controls. No

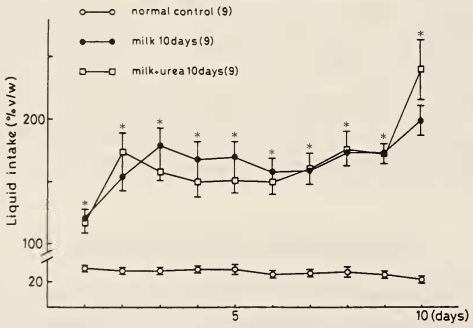


Fig. 1. Daily consumption (% volume/body weight) of distilled water (control), 3% powdered milk in a 5% glucose solution (milk 10 days) and the milk diet supplemented with 1% urea (milk+urea 10 days) in male mice. * $p<0.001$ vs control. Number of animals is in parentheses.

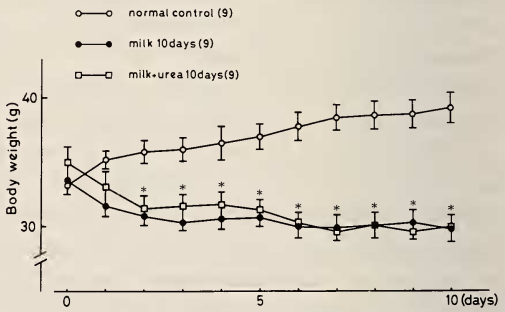


Fig. 2. Body weight of mice given distilled water, the liquid diet (3% milk in 5% glucose) and the urea-containing liquid diet, respectively, for 10 days. * $p<0.001$ vs control.

significant difference was observed between the two groups of mice fed the liquid diet with or without urea (Table 1). No significant difference existed in plasma osmolality values among the control and the two experimental groups (Table 1).

TABLE 1. Hematocrit and plasma osmolality in male mice fed the pelleted food (Control), the liquid diet and the liquid diet supplemented with 1% urea, respectively, for 10 days

Treatment	Hematocrit (%)	Plasma osmolality (mOsmol/kg)
Control (9)	48.7±0.1	301±2.6
Liquid Diet (9)	53.7±0.3*	299±1.5
Liquid Diet+Urea (9)	52.8±0.5*	298±0.9

* $p<0.002$ *vs* control. Number of animals is in parentheses.

3. *Fine structure of the pituitary PI cells*

The PI cells of the control mice contained numerous secretory granules and had occasionally an indented nucleus. The Golgi apparatus was small, and immature granules in the Golgi region were sparse. The rough endoplasmic reticulum (r-ER) was not so well developed (Fig. 3). The PI cells from mice given the liquid diet appeared to be active in fine structure. Large Golgi apparatus and distended cisternae of the r-ER were conspicuous in PI cells of animals fed the liquid diet with urea (Fig. 4).

4. *Morphometry of the pituitary PI cells*

The percent area of the cytoplasm occupied by the r-ER in mice fed the liquid diet without urea, and in animals given the liqud diet supplemented with urea increased to 124% and 172% of the control value, respectively (Fig. 5). The numerical density of Golgi immature granules in mice maintained on the liquid diet, and in those on the liquid diet with urea was 143% and 185% of the control value, respectively (Fig. 5). On the contrary, the numerical density of secretory granules in mice fed the liquid diet, and in animals given on the liquid diet supplemented with urea decreased to 74% and 62% of the control value, respecttively (Fig. 5).

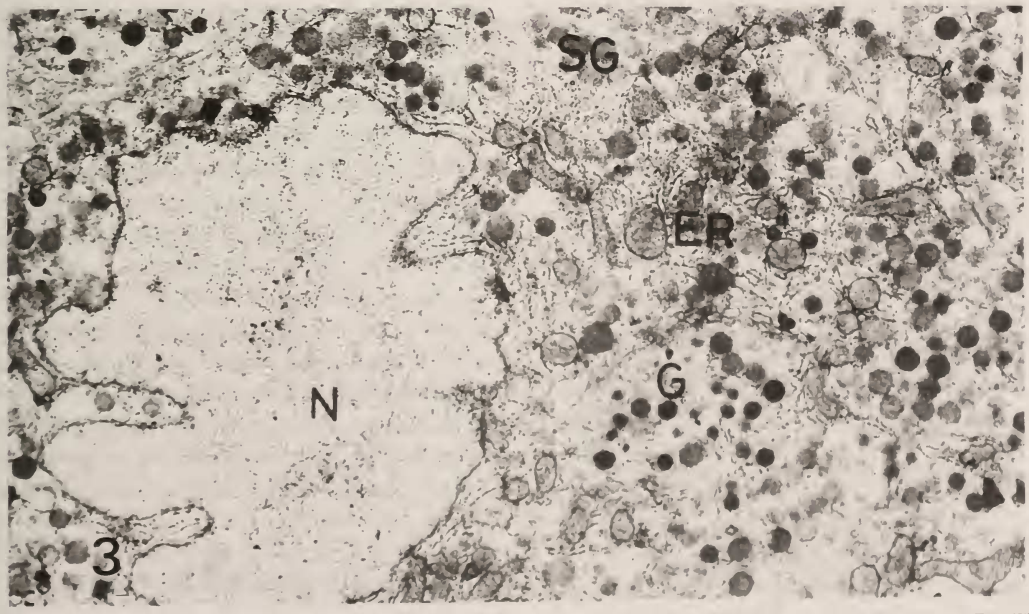


FIG. 3. Part of the pituitary PI cell from control mouse. The cytoplasm is abundant in secretory granules (SG). The rough endoplasmic reticulum (ER) is sparse and its cisternae are short and tubular profiles. The Golgi apparatus (G) is small and is situated in the vicinity of the indented nucleus (N). ×8500.

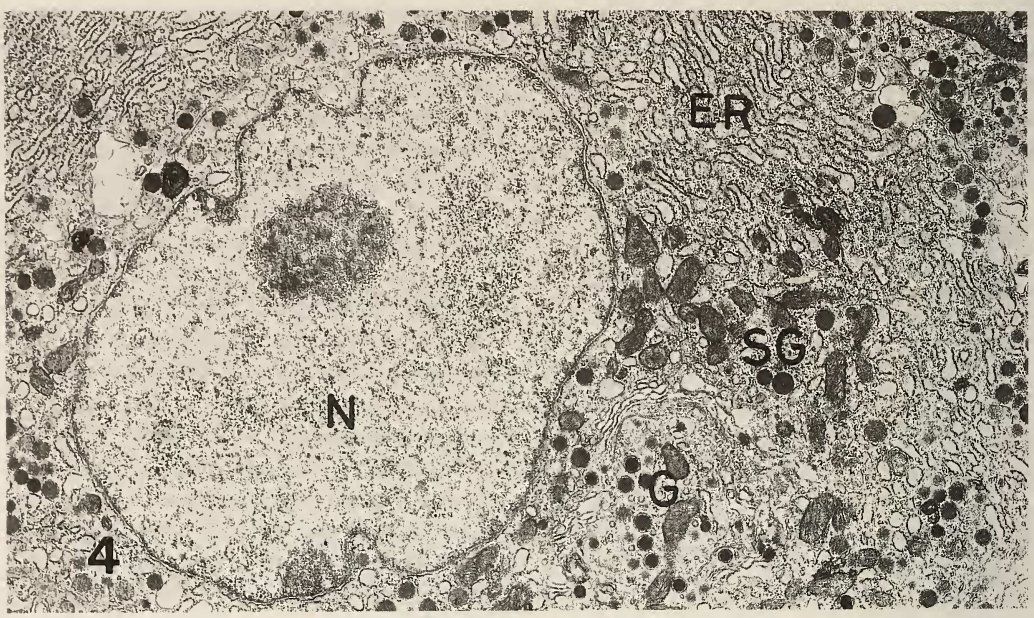
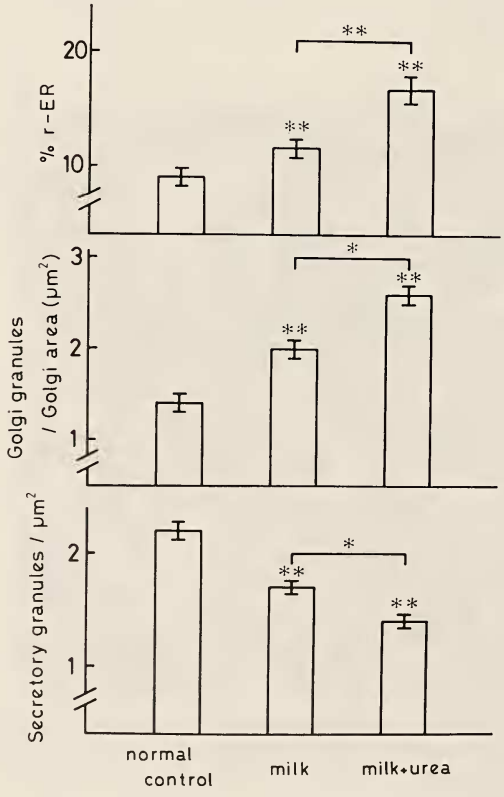


Fig. 4. Part of the pituitary PI cell from mouse fed the liquid diet supplemented with 1% urea for 10 days. Note lamellar rough endoplasmic reticulum (ER), extensive Golgi apparatus (G) and sparse secretory granules (SG). ×8500.



DISCUSSION

The present morphometric study demonstrated that voluntary oral intake of a liquid diet consisting of 3% powdered milk in a 5% glucose solution for 10 days resulted in hypersecretory activity of the pars intermedia (PI) cells of the pituitary in male mice. The effect was significant in animals fed the identical liquid diet supplemented with 1% urea. These findings are consistent with our previous study on pituitary PI cells of lactating mice subjected to the copious drinking of a 5% glucose solution containing 1% urea [5].

Morphometric study at the electron microscopic level is advantageous in evaluating the secretory activity of the pituitary PI cells because the PI cells contain several biologically active peptides drived from proopiomelanocortin [6, 7] and which peptide(s) being released in response to oral urea

Fig. 5. The percent area of the r-ER, the numerical density of Golgi immature granules and of secretory granules in cells of the pituitary pars intermedia from male mice under the conditions indicated at abscissa. * $p<0.01$ *vs* control, ** $p<0.001$ *vs* control.

administration is not known at present. Morphometric changes in the percent area of the rough endoplasmic reticulum (r-ER), and in the numerical density of Golgi immature granules and secretory granules in PI cells reflect quantitatively the degree of protein synthesis, granule formation and release, respectively.

We have proposed a hypothesis that the PI hormones are respensible for the regulation of hydromineral balance in mice [1–4]. Subsequently, *in vitro* studies have confirmed our hypothesis by demonstrating that proopiomelanocortin-derived PI peptides such as α-MSH [8–10], β-MSH [11, 12], and γ-MSH [13–15] at physiological concentrations augmented aldosterone secretion by adrenals.

Although marked hypersecretion of PI cells in mice was induced by dietary sodium deficiency [1, 2] and copious drinking [4], these experimental manipulations are unnatural. Accordingly, we have made some attempts to find relevant physiological or pathological conditions under which the secretory activity of PI cells are expected to be augmented. In this connection, there is a parallelism between the high blood urea concentration and the hypertrophy of the PI in mice with hereditary nephrogenic diabetes inspidus (DI) [16, 17].

An exact causal relationship between exogenous urea and the pituitary PI function remains unclear. Stimulation of PI cells by exogenous urea observed in the present study is probably due to impairment of hypothalamic inhibitory control over the PI, the principal inhibitory factor being dopamine [18]. In a separate experiment, the serum sodium concentration showed no significant change in male mice fed with pelleted food containing 24% urea for 10 days (unpublished data). Thus, oral urea stimulation of PI cells could be mediated by factors other than the low sodium blood level. Neither hematocrit nor plasma osmolality levels could explain the effect of urea on PI cells in mice because there were no significant differences between the two experimental groups.

The present study suggests that the PI hormones may be involved in the regulation of excess blood urea as well as the regulation of hydro-mineral balance stated elsewhere [1–4]. Further studies are now in progress.

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